REMARKS

Status of Claims

Claims 1-21 are allowed. Claims 22, 25 and 26 were previously cancelled.

Claims 23 and 24 depend from claim 1 and should be allowable with it since the above amendment resolves the issues raised in the action.

Claim 29 recites novel, useful and unobvious subject matter, supported in the specification. See also claim 24. The Examiner's objection as to form is met by the amendment of this claim.

These amendments and cancellations of claims are made without prejudice. The specification is enabling as to claims 23, 24 and 29 as now amended.

The goal of vaccination is the generation of strong immune response to the administered antigens able to provide long term protection against infection (See Ref. A appended hereto: Nikolai Petrovsky,"Vaccine adjuvants: Current state and future trends," IMMUNOLOGY AND CELL BIOLOGY, 2004, 82, 488-496, line 1-3).

Adjuvants are compounds that enhance the specific immune response against co-inoculated antigens. Petrovsky, *supra,* 488, lines 5-7).

Par. [0022] of the present application describes adjuvants as substances that enhance immune response to antigens.

Par. [0018] of the present application describes a need for adjuvants and provides an example of use of alum as an adjuvant to hepatitis B antigen to provide prophylaxis against hepatitis B (disease specific to incorporated antigen).

Par. [0024] of the present application describes use of alum as an adjuvant for some vaccines like hepatitis B, diphtheria, tetanus, toxoid etc.

Examples as paras. [0130]-[0133] and [0155]-[0156] and related figures of the present application illustrate achievement of protective titers with use of adjuvants as per the present invention compared to control groups.

The Examples at paras. [0146] to -[0149] clearly show the faster and specific induction of neutralizing antibody response to protective titers in 10 days in Mw group as indicated in Table 2 below from para. [0148] and FIG. 8.

TABLE - 2

Effect of immunization with Immuvac™ Mw in mice		
	Rabies control vaccine	Rabies vaccine Mw
Day 5	Not Detectable	Not Detectable
Day 10	0.2975	1.35
Day 25	1.09	32.00

An effect of 0.5 is considered protective titer for rabies as per World Health Organization (WHO) standards (See,Refs.:B-E appended hereto):. "Rabies Postexposure Prphylaxis with Human Diploid Cell rabies Vaccine: Lower Neutralizing Antibody titers with Wyeth Vaccine," from Centers for Disease Control, MMWR Weekly cdc.gov/mmwr/.../00000487.htm, February 22, 1985, 34(7);90-2)

C. "Rabies Vaccine IMOVAX RABIES, wistar rabies virus strain PM-1503-3M grown in human diploid cell cultures," Product Information of Sanofi Pasteur SA (Dec. 2005)

D. RABAVERT – "Rabies virus inactivated antigen, an injection, powder, lyophilized, for suspension) Product information," Novartis Vaccines and Diagnostics Gmbh & Co. KG

E. USFDA approved label of RABAVERT and IMOVAX download on 4th September 2010 as well as MMWR, February 22, 1985/34(7);90-2.

In view of the amendments presented and the foregoing remarks, Applicants believe that the amended claims added to the present application are now in compliance with 37 C.F.R. §1.112 and all other patentability requirements. Therefore, Applicants respectfully request a favorable action allowing claims 1-21, 23, 24 and 29.

The Director of the U.S. Patent and Trademarks Office is authorized to charge any deficiencies, or to credit any overpayments, to Deposit Account No. 03-2410; Attorney Docket No.: 81094-00004.

If any further submission by Applicants herein or their response to any questions would be helpful to the Examiner a telephone conference is encouraged.

The following information is presented in the event that a call may be deemed desirable by the Examiner:

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Respectfully submitted,
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Dated: September 7, 2010

/Jerry Cohen/
Jerry Cohen, Reg. No.: 20,522

Enclosures: Power of Attorney, Request for One Month Extension of Time
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Special Feature

Vaccine adjuvants: Current state and future trends

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Summary The problem with pure recombinant or synthetic antigens used in modern day vaccines is that they are generally far less immunogenic than older style live or killed whole organism vaccines. This has created a major need for improved and more powerful adjuvants for use in these vaccines. With few exceptions, alum remains the sole adjuvant approved for human use in the majority of countries worldwide. Although alum is able to induce a good antibody (Th2) response, it has little capacity to stimulate cellular (Th1) immune responses which are so important for protection against many pathogens. In addition, alum has the potential to cause severe local and systemic side-effects including sterile abscesses, eosinophilia and myofascitis, although fortunately most of the more serious side-effects are relatively rare. There is also community concern regarding the possible role of aluminium in neurodegenerative diseases such as Alzheimer's disease. Consequently, there is a major unmet need for safer and more effective adjuvants suitable for human use. In particular, there is demand for safe and non-toxic adjuvants able to stimulate cellular (Th1) immunity. Other needs in light of new vaccine technologies are adjuvants suitable for use with mucosally-delivered vaccines, DNA vaccines, cancer and autoimmunity vaccines. Each of these areas are highly specialized with their own unique needs in respect of suitable adjuvant technology. This paper reviews the state of the art in the adjuvant field, explores future directions of adjuvant development and finally examines some of the impediments and barriers to development and registration of new human adjuvants.

Key words: adjuvants, immune response, mucosal immunity, vaccines.

Adjuvant origins

The goal of vaccination is the generation of a strong immune response to the administered antigen able to provide long-term protection against infection. To achieve this objective with killed as opposed to live attenuated vaccines, often requires the addition of an adjuvant.[1] Adjuvants are compounds that enhance the specific immune response against co-inoculated antigens. The word adjuvant comes from the Latin word *adjuvare*, which means to help or to enhance.[2] The concept of adjuvants arose in the 1920s from observations such as those of Ramon *et al.* who noted that horses that developed an abscess at the inoculation site of diphtheria toxoid generated higher specific antibody titres.[3,4] They subsequently found that an abscess generated by the injection of unrelated substances along with the diphtheria toxoid increased the immune response against the toxoid.[3,4] The adjuvant activity of aluminium compounds was demonstrated by Glenny *et al.* in 1926 with diphtheria toxoid absorbed to alum.[5] To this day, aluminium-based compounds (principally aluminium phosphate or hydroxide) remain the predominant human adjuvants.[6] In 1936, Freund developed an emulsion of water and mineral oil containing killed mycobacteria, thereby creating one of the most potent known adjuvants, Freund's complete adjuvant (FCA).[7,8] Despite being the gold standard adjuvant, FCA causes severe local reactions and is considered too toxic for human use. The oil in water emulsion without added mycobacteria is known as Freund's incomplete adjuvant (FIA) and, being less toxic, has been used in human vaccine formulations.[8] In the 1950s, Johnson *et al.* found that lipopolysaccharides (LPS) from Gram-negative bacteria exhibited adjuvant activity[9] and detoxified LPS or related compounds such as lipid A have since been used as adjuvants in human studies.[10] In 1974, Lederer *et al.* identified muramyldipeptide (MDP) as a mycobacterial component with adjuvant activity contained in FCA.[11] Bacterial components are often potent immune activators although commonly associated with toxicity, for example, bacterial DNA with immunostimulatory CpG motifs is one of the most potent cellular adjuvants.[12] Immunostimulatory CpG are unmethylated cytosine-guanine dinucleotides found in bacterial DNA but absent in mammalian DNA. Overall, several hundred natural and synthetic compounds have been identified to have adjuvant activity. Although a significant number are clearly more potent than alum, toxicity is perhaps the single most important impediment in introducing most such adjuvants to human use.[2]

Adjuvant roles

Adjuvants can be used for various purposes: (i) to enhance the immunogenicity of highly purified or recombinant antigens; (ii) to reduce the amount of antigen or the number of immunizations needed for protective immunity; (iii) to improve the efficacy of vaccines in newborns, the elderly or immunocompromised persons; or (iv) as antigen delivery systems for the uptake of antigens by the mucosa.[13–15]

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Adjuvant selection

Some of the features involved in adjuvant selection are: the antigen, the species to be vaccinated, the route of administration and the likelihood of side-effects.[16,17] Ideally, adjuvants should be stable with long shelf life, biodegradable, cheap to produce, not induce immune responses against themselves and promote an appropriate immune response (i.e. cellular or antibody immunity depending on requirements for protection).[18] There are marked differences in the efficacy of adjuvants depending on the administration route (e.g. between mucosal and parenteral routes). Hence new vectors, antigen delivery systems or adjuvant compounds need to take into account the characteristics of the proposed administration route.[19]

Adjuvant safety issues

The benefits flowing from adjuvant incorporation into any vaccine formulation have to be balanced with the risk of adverse reactions.[20,21] Adverse reactions to adjuvants can be classified as local or systemic. Important local reactions include pain, local inflammation, swelling, injection site necrosis, lymphadenopathy, granulomas, ulcers and the generation of sterile abscesses. Systemic reactions include nausea, fever, adjuvant arthritis, uveitis, eosinophilia, allergy, anaphylaxis, organ specific toxicity and immunotoxicity (i.e. the liberation of cytokines, immunosuppression or autoimmune diseases).[22,23] Unfortunately, potent adjuvant action is often correlated with increased toxicity, as exemplified by the case of FCA which although potent is too toxic for human use. Thus, one of the major challenges in adjuvant research is to gain potency while minimizing toxicity. The difficulty of achieving this objective is reflected in the fact that alum, despite being initially discovered over 80 years ago, remains the dominant human adjuvant in use today.

Adjuvant regulatory requirements

Regulations for the human use of adjuvants are far more rigorous than those applied to veterinary vaccines. In addition to preclinical studies on the adjuvant itself, the combined antigen-adjuvant formulation also needs to be subjected to toxicology prior to commencement of phase 1 clinical trials.[24] The toxicological evaluation is normally conducted in small animal species such as mice, rats or rabbits and should use the same administration route proposed for human use. The dose and frequency of vaccination for preclinical toxicology should be similar to or higher than the proposed human dose to maximize the ability to identify potential safety problems.[25] Preclinical studies may also help in selecting the optimum vaccine dose.[26]

Adjuvant classification

Adjuvants can be classified according to their source, mechanism of action or physicochemical properties.[2] Edelman[22] classified adjuvants into three groups: (i) active immunostimulants, being substances that increase the immune response to the antigen; (ii) carriers, being immunogenic proteins that provide T-cell help; and (iii) vehicle adjuvants, being oil emulsions or liposomes that serve as a matrix for antigens as well as stimulating the immune response. An alternative adjuvant classification divides adjuvants according to administration route, namely mucosal or parenteral. A third classification divides adjuvants into alum salts and other mineral adjuvants; tensoactive agents; bacterial derivatives; vehicles and slow release materials or cytokines.[17] A fourth more recently proposed system of classification divides adjuvants into the following groups: gel-based adjuvants, tensoactive agents, bacterial products, oil emulsions, particulated adjuvants, fusion proteins or lipopeptides.[27]

Adjuvant limitations

In spite of progress in the identification of mechanisms of adjuvant action, alum remains the dominant adjuvant for human vaccines. Although many other adjuvants have been proposed over the years, these have failed to be successful in humans largely because of toxicity, stability, bioavailability and cost. Because of effects of size, electric charge and hydrophobicity which regulate the incorporation of proteins into the adjuvant formulation, it is difficult to predict on an empirical basis which adjuvant will work most effectively with a particular protein or peptide. Moreover, epitope modifications may occur during formulation or conjugation. In the case of carrier proteins, a pre-existing immunity to the carrier protein is a major limitation.[27] Furthermore, each adjuvant generates a characteristic immune response profile. For example, the inability of alum-based adjuvants to induce Th1 antibody isotypes or cellular immune responses, and their poor adjuvant effect on polysaccharide antigens limit their applicability to many vaccines.[16]

Major adjuvant groups

Mineral salt adjuvants

Alum-based adjuvants

Since the experiments of Glenny *et al.*[5] alum salts, principally aluminium phosphate or hydroxide, have been the most widely used human adjuvants.[16] Unfortunately, alum salts are relatively weak adjuvants and rarely induce cellular immune responses.[28-30] Studies suggest alum salts work by causing the formation of an antigen depot at the inoculation site from where antigen is released slowly.[31] The trapping of soluble antigen in the alum gel may also increase the duration of antigen interaction with the immune system. Other mechanisms of action involve complement, eosinophil and macrophage, activation[32] and increased efficiency of antigen uptake by antigen presenting cells seen with particulate matter with a size under 10 μm.[33]

Whilst alum-based vaccines are generally well tolerated, granulomas are common when the subcutaneous or intradermal route is used rather than intramuscular injection.[34,35] Other specific limitations of alum adjuvants are increased IgE production, allergenicity[30,33,34,36-38] and neurotoxicity. Although under normal circumstances low doses of aluminium are excreted by the kidneys, under certain conditions such as reduced renal function, aluminium is accumulated in the body and becomes highly toxic. High aluminium levels in the body predominately affect the brain and bone tissues causing

fatal neurological syndrome and dialysis-associated dementia. Aluminium intoxication has also been associated with amyotrophic lateral sclerosis and Alzheimer's disease.

Other mineral salt adjuvants

The salts of calcium, iron and zirconium have also been used to adsorb antigens, although not to the extent of alum salts.[34] In particular, calcium phosphate has been used for diphtheria-tetanus-pertussis vaccines.[39,40] While having similar properties to alum salts, calcium phosphate has the advantage that it is a natural compound to the human body and is therefore exceptionally well tolerated. It has a reasonable capacity to adsorb antigens, induces high levels of IgG antibodies and does not increase IgE production. Neurological reactions to pertussis vaccines adsorbed to calcium phosphate are rare.[41]

Tensoactive adjuvants

Quil A is a saponin derived from an aqueous extract from the bark of *Quillaja saponaria*. Fractions purified from this extract by reverse phase chromatography, mainly QS-21, have been studied as alternatives to alum when strong cellular responses are required for a particular vaccine.[22,42,43] Saponins are tensoactive glycosides containing a hydrophobic nucleus of triterpenoid structure with carbohydrate chains linked to the nucleus.[42] Saponins induce a strong adjuvant effect to T-dependent as well as T-independent antigens. Saponins also induce strong cytotoxic CD8+ lymphocyte responses and potentiate the response to mucosal antigens.[42] Quil A has been used successfully for veterinary applications.[44] It is a natural product composed of more than 23 different saponins and is generally considered too toxic for human use. In addition to severe local reactions and granulomas, toxicity includes severe haemolysis reflecting the affinity of saponins for cholesterol present in erythrocyte membranes, resulting in membrane solubilization and haemolysis.[20,44–47] The Quil A-derived saponin QS-21, whilst less toxic than Quil A itself, has many of the same problems and may similarly prove unsuitable for most human uses other than therapeutic vaccines for life threatening illnesses such as HIV infection.[48]

Bacteria-derived adjuvants

Given their potent immunostimulatory capacity, bacteria-derived substances constitute a major potential source of adjuvants. Cell wall peptidoglycan or lipopolysaccharide of Gram-negative bacteria, enhance the immune response against co-administered antigens despite themselves not being very immunogenic. This adjuvant activity is mediated through activation of Toll-like receptors that mediate the danger signals activating the host immune defence system.[35] Different species of bacteria used as a source of adjuvants include *Mycobacterium* spp., *Corynebacterium parvum*, *C. granulosum*, *Bordetella pertussis and Neisseria meningitidis*. Unfortunately, as whole alive or killed microorganisms these are too toxic to be used as human adjuvants.[23] However, much of the adjuvanticity of these bacteria is mediated by N-acetyl muramyl-L-alanyl-D-isoglutamine, also called MDP. The adjuvanticity of MDP depends on the administration conditions.[11,49] In saline, it mainly enhances humoral immunity[20,50,51] whilst when incorporated into liposomes or mixed with glycerol it induces strong cellular immunity.[52] MDP activates many different cell types including macrophages, leucocytes, mastocytes, endothelial cells and fibroblasts inducing the secretion of cytokines such as IL-1, B-cell grow factor and fibroblast activating factor. MDP also induces an increase in the production of superoxides, prostaglandins and collagenase.[53] Different compounds derived from MDP include threonyl-MDP, a potent yet non-pyrogenic adjuvant.[23]

Another important group of compounds derived from the cell wall of Gram-negative bacteria are the lipopolysaccharides (LPS). They are potent B-cell mitogens, but also activate T cells to produce IFN-γ and TNF and thereby enhance cellular immune responses. The major structural element responsible for their toxicity and adjuvant effect is Lipid A. In low acid conditions, lipid A can be hydrolysed to obtain monophosphoryl lipid A, a compound which retains the adjuvant activity of Lipid A with reduced toxicity.[54] Another extract from bacterial walls is trehalose dimycolate (TDM), an adjuvant which simulates both humoral and cellular responses.[55] The demonstration that mycobacterial DNA had adjuvant activity, led to the discovery that the adjuvant activity correlated with a higher content of CpG motifs present in bacterial nucleic acids. DNA containing CpG motifs is one of the most potent cellular adjuvants.[12]

Adjuvant emulsions

This class includes oil in water or water in oil emulsions such as FIA, Montanide, Adjuvant 65, and Lipovant.[17] The mechanism of action of adjuvant emulsions includes the formation of a depot at the injection site, enabling the slow release of antigen and the stimulation of antibody producing plasma cells.[56] In general, these adjuvants are too toxic for routine human prophylactic vaccine use, although they may be suitable for use in terminal conditions such as cancer where there is a greater tolerance of side-effects. Frequent side-effects of emulsions include inflammatory reactions, granulomas and ulcers at the injection site. Various types of emulsions have been used, with different natural oils, in order to find more stable, potent and less toxic formulations. Adjuvant 65 offers the advantage over the mineral oil used in IFA[57–59] that it can be metabolized.[23] Different emulsions like oil in water[60] and water in oil in water[61] have been developed with the latter being as potent as IFA but more stable, less viscous and easier to administer with less resulting granulomas.[62,63] Montanide is a family of oil-based adjuvants that have been used in experimental vaccines in mice, rats, cats and dogs, using natural, recombinant and synthetic antigens. In humans, Montanide has been used in trial vaccines against HIV, malaria and breast cancer.[64]

Liposome adjuvants

Liposomes are synthetic spheres consisting of lipid layers that can encapsulate antigens and act as both a vaccine delivery vehicle and adjuvant. Liposomes have been used widely in experimental vaccines. The potency of liposomes depends on the number of lipid layers,[65] electric charge,[66] composition[67] and method of preparation.[67–69] They enhance both humoral and cellular immunity to protein and polysaccharide antigens.[66,69]

Liposomes help extend the half-life of antigens in blood ensuring a higher antigen exposure to antigen presenting cells after vaccination.[70] Stability, manufacturing and quality assurance problems seem to have been major factors behind the fact that as yet no adjuvant based on liposomes has been registered for human use.

Polymeric microsphere adjuvants

Among particulated and polymeric systems, poly (DL-lactide-coglycolide) microspheres have been extensively studied. These are biocompatible and biodegradable microspheres able to incorporate different antigens. One of the advantages of this system is the capacity to manipulate the degradation kinetics of the microspheres by varying the relative concentration of their components, thereby controlling the time of antigen release.[71,72]

Cytokines as adjuvants

Cytokines are included in the modern classification of adjuvants. IFN-γ is a pleiotropic cytokine able to enhance cellular immune responses through a variety of mechanisms.[73] Granulocyte-macrophage colony stimulating factor (GM-CSF) enhances the primary immune response by activating and recruiting antigen presenting cells.[74] The practical application of GM-CSF as an adjuvant has been limited by the requirement for multiple doses, toxicity and the immunogenicity of heterologous cytokines.[12] Cytokines are particularly seen to have potential in DNA vaccines where the cytokine can be expressed by the same vector as the antigen.

Carbohydrate adjuvants

Inulin-derived adjuvants

Several complex carbohydrates of natural origin stimulate cells from the immune and reticulo-endothelial system.[75] The main source of these polysaccharides have been plants and fungus. Gamma inulin, a carbohydrate derived from plant roots of the Compositae family, is a potent humoral and cellular immune adjuvant. Gamma inulin is a potent alternate complement pathway activator increasing production of activated C3 and thereby activating macrophages.[76] Gamma inulin is effective at boosting cellular immune responses without the toxicity exhibited by other adjuvants such as FCA. Gamma inulin can be combined with a variety of other adjuvant components, for example, aluminium hydroxide, to produce a range of tailor made adjuvants with varying degrees of Th1 and Th2 activity. For example, Algammulin is a combination of γ-inulin and aluminium hydroxide. Algammulin exhibits a higher ratio of Th2 to Th1 activity than γ-inulin alone, its overall effect being equivalent to alum despite having a lower overall alum content.[77,78] Inulin-based adjuvants have successfully been tested in multiple animal models in combination with such antigens as diphtheria and tetanus toxoid, respiratory syncytial virus, the E7 protein from the Human Papilloma Virus, Herpes Virus 2 glycoprotein D, Hepatitis B surface antigen, influenza haemagglutinin, Haemophilus influenzae antigens and antigens from *Plasmodium falciparum*. Major advantages of inulin-derived adjuvants are that they induce both Th1 and Th2 immune responses, unlike alum do not induce IgE, and are not associated with any significant local or systemic toxicity.[79] Inulin is metabolisable into simple sugars fructose and glucose. Inulin does not, therefore, suffer from concerns regarding long-term accumulation and toxicity that are associated with metal-based compounds such as alum.

Other carbohydrate adjuvants

Polysaccharides based on glucose and mannose which have adjuvant action include glucans, dextrans, lentinans, glucomannans and galactomannans. Levans and xylans,[80] (82) also have immuno-enhancing activity. Macrophages have glucan and mannan receptors, activation of which stimulates phagocytosis and cytokine secretion plus release of leukotrienes and prostaglandins. Polysaccharides have been used for immune stimulation in patients with cancer.[81] *In vitro*, mannan activates monocytes and macrophages to secrete IFN, TNF, GM-CSF, IL-1 and IL-6.[82] Acemannan, a natural polysaccharide extracted as a mucilaginous gel of the *Aloe barbadensis*, stimulates generation of cytotoxic T lymphocytes (CTL)[83] and the cytotoxic activity of NK cells.[84] Recently, acemannan has been shown to enhance the immune response to nasally administered Hepatitis B surface antigen (HBsAg), generating similar levels of IgG antibody titres in sera compared to the immune response generated by an intramuscular alum-based HBsAg control vaccine.[85]

Adjuvant formulations

New adjuvant formulations have resulted from the mixture of different adjuvants in the same formulation. As a general rule, two or more adjuvants with different mechanisms of action are combined to enhance the potency and type of the immune response to the vaccine antigen. For example, alum salts can be formulated in combination with other adjuvants such as Lipid A to increase immunogenicity. Similarly algammulin which is the combination of γ-inulin plus alum has increased absorptive capacity and increased ability to stimulate Th2 responses.[77] Saponins such as Quil A have also been used as a part of immunostimulatory complexes (ISCOMS).[42] ISCOMS are virus like particles of 30-40 nm and dodecahedric structure, composed by Quil A, lipids and cholesterol. Antigens can be inserted in the membrane or encapsulated. A wide variety of proteins have been inserted in these cage-like structures.[86-88] ISCOMS can be used through the oral, respiratory and vaginal routes.[89] ISCOMS are particularly effective in activating cellular immunity and cytotoxic T cells[42] but often have problems with stability and toxicity.

Mucosal adjuvants

The development of adjuvants for mucosal immunization is an important current area of vaccine development. The quality of mucosal adjuvants needs to take into account the ability to stimulate the uptake of antigen through the various mucosal routes, and its ability to enhance the immunogenicity of mucosally-delivered antigen. Different results can be obtained for the same adjuvant when administered by a parenteral or mucosal route. Alum salts, the most widely used parenteral adjuvants, are ineffective when administered by the oral or

nasal route.[90] The mucosa is a door of entry for many pathogens. Although it is very difficult to generate mucosal antibodies through parenteral vaccination, it is possible to obtain mucosal as well as parenteral immunity by inoculating antigen by the mucosal route.[91] For pathogens colonizing mucosal surfaces or those having a mucosal route of entry, protection correlates well with a strong local mucosal response.[92] For mucosal immunization, several adjuvant strategies involve binding or coating with specific ligands to deliver the antigens to specialized epithelial cells (M cells). It is also important to correctly match the physicochemical characteristics of the antigen-like size, electric charge, and hydrophobicity to let the antigen cross mucosal barriers.[19] After optimization of these characteristics, the selected adjuvant may also enhance the immune response by mechanisms already described: adsorption and depot effect, cytokine induction, complement activation, recruiting of different cell populations, the delivery to different APC, the regulation of the expression via MHC class I or class II and the stimulation of the production of different subtypes of antibodies.[93]

Bacterial derivatives

Some well known parenteral adjuvants, like MDP, monophosphoryl lipid A (MPL) and LPS, also act as mucosal adjuvants. Compounds like bacterial toxins of *Vibrio cholerae* (CT) and *Escherichia coli* (HLT) and their respective toxoids are particularly useful mucosal adjuvants.[94-96] Although CT remains one of the most potent known mucosal adjuvants, it suffers from high toxicity and also induces a strong immune response against itself. The strong adjuvanticity of CT and HLT may be explained by their ability to increase antigen presentation by B cell, B-cell differentiation to IgA secreting cells, interaction with T cells and increase cytokine production.[97] The B subunit of CT is less toxic and strategies have been taken to mutate the gene coding for CT in order to detoxify the cholera toxin.

Synthetic or inactivated antigen delivery systems

This group of mucosal adjuvants includes different synthetic polymeric particles composed by biodegradable poly(DL-lactide-co-glycolide) (DL-PLG), cellulose acetate, iminocarbonates, proteinoid microspheres, polyanhydrides, dextrans, as well as particles produced from natural materials like alginates, gelatine and plant seeds. Other natural compounds like liposomes, virosomes and ISCOMS can also be included in this group.[98] Particle size is one of the major factors involved in the mucosal delivery. It has been shown that particles over 10 μm are not adsorbed by the intestinal mucosa.[99] Lower size particles can be taken up by Peyer's patches, and those lower than 1 μm can penetrate to lymph nodes and the liver, and reach the circulatory system.[100,101] Liposomes, cochleates and microparticles can bind mucosal surfaces by hydrophobic interactions, but their entry to M cells is not efficient because they are rapidly trapped in mucosal gels and most of them are not able to reach the mucosa. Macromolecules or particles conjugated or covered by ligands such as cholera toxin b chain (CTB) are limited by their need to gain accessibility to specific receptors.[102] The balance between hydrophobicity-hydrophilicity for the antigen delivery systems can be modified to obtain a modulation in the immune response.[100] The use of ligands linked to particles can result in the specific adherence to M cells, but only in a size range restricted by the glycocalix. Particles of 1 μm or higher require targeting of ligands to M cells.[103]

Living antigen mucosal delivery systems

Some pathogenic bacteria have the ability to overcome the difficulties of non-living systems in being uptaken easily by specific M cell receptors. One example is attenuated *Salmonella typhi* ty21a, which has a lectin-like interaction with polysaccharide receptors on M cells. Also *V. cholerae and* poliovirus strains can be used for oral immunization of heterologous antigens. Genetically modified strains of these microorganisms have been used as a carrier of heterologous antigens.[104] The biology of these living vectors introduces new challenges. *V. cholerae* vaccine strains without the genes coding for their toxins remain toxic.[104] Pre-existing immune responses in a high number of subjects previously immunized naturally or by vaccination constitutes a major drawback for this strategy.

Cytokines

High doses of IFN-α abrogate oral tolerance.[105] Similar results have been obtained with IL-12.[106] This suggests that orally administered cytokines may be able to be used as mucosal adjuvants to overcome systemic immune unresponsiveness, for example that seen in chronic Hepatitis B infection.

Adjuvants for DNA immunization

When naked DNA immunization commenced in the 1990s, it was supposed that this kind of immunogen would not need adjuvants. It is now clear that novel strategies are required to enhance the potency of DNA-based vaccine candidates. The strategy of co-inoculating plasmids coding for different cytokines or costimulatory factors to enhance the immune response generated by the vaccine plasmid has been used successfully.[107] Co-inoculation of the plasmid expressing B7-2 along with a DNA vaccine candidate from HIV-1, increased the cellular immune response specific for HIV-1. Also a plasmid expressing GM-CSF boosted the humoral immune response to protein G from rabies virus when two plasmids coding for each protein were co-inoculated.[73] IL-12 expressing plasmid co-inoculated along with another plasmid coding for an HIV-1 protein enhanced the cell mediated immunity specific for VIH-1.[108]

DNA vaccines and particulate adjuvant systems

Polymers and particulate systems have been used in the field of DNA immunization. Polylactic microspheres, polycarbonates and polystyrene particles about 1 μm in size have been used mucosally and parenterally, resulting in better results compared to free DNA administration.[109] The use of mannans covering polymers of N t-butyl N' tetradecylamin-propionamidine (diC14 amidine), have been used as an immunoenhancing strategy for DNA vaccination. The main effects caused by the co-administration of these structures with DNA is the increase in DTH and CTL responses.

DNA vaccine immunomodulators of cancers have been used in DNA immunization. Ubenimex (UBX) increased humoral and cellular responses to DNA vaccination.[110] The

immune response against DNA encoded antigens has been evaluated nasally and parenterally using immunomodulators already used with protein antigens like MPL and saponins.[111] The response found after intramuscular inoculation of DNA in PBS have been explained in part by the 'danger signal' offered by the DNA itself. Immunostimulatory sequences from procariotic DNA are able to induce several cytokines like IL-12, TNF and IL-6, and thereby have an adjuvant action.[112]

Cancer vaccine adjuvants

There is increasing excitement regarding the potential for anti-cancer vaccines to slow or even eradicate some tumours.[113,114] These vaccines utilize either complete tumour cells, tumour antigens or tumour growth factor receptors combined with powerful adjuvants. These vaccines, being based on self molecules are generally of very low immunogenicity thereby leading to the requirement for potent adjuvants for effect. Approaches taken include the use of Montanide adjuvants, very small size proteoliposomes (VSSP) obtained form the external membrane of *Neisseria meningitidis*[115] or the use of peptides adjuvated with GM-CSF.[116]

Conclusions

Despite an explosion of knowledge regarding immune function over recent decades, we remain almost totally reliant for human adjuvants on aluminium-based compounds whose activity was first discovered over 80 years ago. Recent advances in vaccine development and, in particular, the increasing use of recombinant subunit and synthetic vaccines makes the need for improved adjuvants all the more acute. Although there are glimmers of hope that new adjuvants may rectify some of the deficiencies of aluminium-based adjuvants, there remains a concern that many of these promising adjuvants will never be approved for human use for logistical or commercial rather than scientific reasons. Clearly there are some major barriers other than just a lack of scientific knowledge of adjuvants that are standing in the way of availability of new adjuvants. First and foremost, unacceptable side-effects and toxicity preclude the use of many candidate adjuvants and this is particularly true for prophylactic paediatric vaccines where safety issues are paramount. Second, the regulatory bar has been raised significantly since the days when alum was first introduced as a human adjuvant. Indeed, it is likely that if alum hadn't been in use all these years and was first put forward to regulatory bodies for approval today, it would be refused registration on the basis of safety concerns. Third, it is not possible for adjuvants to be approved as products in their own right as they can only be registered as part of a vaccine combination. It is possible that many good adjuvant candidates have failed to reach the registration phase, not because there were any problems with the adjuvants themselves, but because the vaccine combination was not effective or had toxicity. This could be seen as analogous to throwing out the baby with the bath water. Fourth, having invested considerable funds in the development of a new vaccine antigen, few companies are prepared to risk this investment by conducting clinical trial program of candidate antigens with a new and unproven adjuvant as this could bring the whole development program unstuck if there turned out to be problems with the adjuvant. Fifth, most vaccine companies choose to keep their proprietary adjuvant data secret and therefore until such time as they themselves wish to register a vaccine product based on their adjuvants, then they will not share their knowledge of these adjuvants with others. Finally, the cost of developing a new product such as an adjuvant is now prohibitive. Whilst it might be possible to justify an investment of several hundred million dollars on a new vaccine given the prospect of recovering this investment from vaccine sales, the same does not hold true for adjuvant development costs, for which there is no easy source of cost recovery. For all the above logistical and commercial reasons there is a continuing major unmet need for safe and non-toxic adjuvants, particularly for adjuvants capable of strongly boosting cellular immune responses which are not associated with undue toxicity. Despite many advances of immunology, this key objective remains the 'holy grail' of vaccinology. Hence, the importance of major public institutions such as the NIH and WHO and charities with interests in vaccine development such as the Gates Foundation to fund adjuvant research and development programs as part of their general support for vaccine development.

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Weekly

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Rabies Postexposure Prophylaxis with Human Diploid Cell Rabies Vaccine: Lower Neutralizing Antibody Titers with Wyeth Vaccine

On February 16, 1985, Wyeth Laboratories recalled Wyeth human diploid cell rabies vaccine (WYVACTM) from the market. This resulted from two postlicensure studies of antibody responses after postexposure prophylaxis with human diploid cell rabies vaccine (HDCV) conducted by CDC over the last 6 months. The studies--one, a passive surveillance system, and the other, a randomized prospective study--demonstrated that not all individuals receiving postexposure prophylaxis with Wyeth Laboratories' HDCV had antibody titers acceptable by the CDC criterion* and that antibody titers after rabies postexposure prophylaxis with Wyeth HDCV were lower than those with Merieux HDCV (IMOVAXTM).

In the passive surveillance system, sera were examined from 39 persons (in four states) who had completed postexposure prophylaxis with rabies immune globulin (RIG) and five doses of HDCV; 22 had been vaccinated with Merieux vaccine, and 17, with Wyeth vaccine. Two of the 17 Wyeth vaccine recipients had an inadequate titer by the CDC criterion (1,2); one had no detectable titer. Three additional persons had low titers (acceptable by CDC's criterion but not by the World Health Organization's criterion). In contrast, all 22 recipients of Merieux vaccine had adequate titers by both criteria.

The reason for some low responses after postexposure administration of Wyeth HDCV is unknown. The product has consistently met all applicable release standards, and the failures could not be attributed to a single vaccine lot. Certain host factors may have contributed to the poor response. The median age of the five poor responders to Wyeth vaccine was 42 years, compared with 21 years for the responders. One low responder was a 42-year-old person with epilepsy on chronic phenytoin therapy; phenytoin has inhibitory effects on some immune functions (3). The individual who showed no detectable neutralizing antibody after prophylaxis with Wyeth vaccine was a healthy but obese (6 ft., 275 lbs.) 32-year-old male who received all injections in the buttocks. Two of the three low responders also received their vaccine in the buttocks.

While the surveillance program was being conducted, a prospective study was undertaken. The study participants received rabies postexposure prophylaxis of RIG with five doses of either Merieux or Wyeth vaccine of similar potencies. Titers in the Merieux group were significantly higher (Table 1), although all

persons in both groups had acceptable titers 2-4 weeks after completing prophylaxis (4). Reported by C Langkop, R Martin, DVM, Illinois Dept of Public Health; M Catalano, MD, Montefiore Hospital and Medical Center, New York City, A Porter, Southampton, C Trimarchi, New York State Dept of Health; J Jarvis, Emory University School of Medicine, Atlanta, E Weir, T McKinley, RK Sikes, DVM, State Epidemiologist, Georgia Dept of Human Resources; D Zeidner, MD, A Bowman, J Dennehy, MD, C Rudy, G Stover, R Leipold, MD, T Royer, MD, M Ryan, MD, G Stover, S Toor, MD, T Martin, MD, Geisinger Medical Center, Danville, J Maksimak, MD, RH Kaiser, MD, G Lattimer, MD, M Hart, C Sinner, Divine Providence Hospital, Williamsport, B Jones, DVM, E Witte, VMD, C Hays, MD, State Epidemiologist, Pennsylvania State Dept of Health; Office of Biologics, Research, and Review, Center for Drugs and Biologics, US Food and Drug Administration; Div of Viral Diseases, Center for Infectious Diseases, Div of Field Svcs, Epidemiology Program Office, CDC.

Editorial Note

Editorial Note: Annually, approximately 20,000 people receive rabies postexposure prophylaxis with HDCV in the United States (5). Since the early 1980s, when duck embryo vaccine was replaced by the more immunogenic HDCV, no person has developed rabies after having received the recommended postexposure prophylaxis of RIG and vaccine. Until the current report, data showed that Wyeth HDCV administered intramuscularly induced acceptable antibody levels.

The present low responses in some individuals may be due to both intrinsic differences in the two vaccines and accompanying host factors. Wyeth HDCV is a subunit vaccine, disrupted with tri-(n)butyl phosphate and further inactivated with beta-propiolactone, while Merieux HDCV is a whole virus vaccine inactivated with beta-propiolactone. Other factors, including older age, receipt of mildly immunosuppressive medications and administration of the vaccine into the buttocks, may also have contributed to the lower responses. Injections in the gluteal region will almost always be delivered into fat (6). It is not known whether there is a difference in absorption of the two types of HDCV when administered by this route. It has recently been recognized that administration of hepatitis B vaccine in the gluteal area probably results in a poorer response than vaccination in the deltoid (7). It is recommended that all adult immunizations be administered in the deltoid region (8,9); the deltoid area is the preferred site for HDCV vaccination. The gluteal area remains an acceptable site for large volumes of RIG. HDCV and RIG should never be administered in the same anatomic sites.

One 1.0-ml intramuscular booster with Merieux HDCV in the deltoid area is recommended, based on review of available information, for all persons who have been potentially exposed to rabies since October 15, 1984, and who have received postexposure prophylaxis with Wyeth HDCV (unless sera obtained after postexposure prophylaxis demonstrated an acceptable antibody titer). Merieux HDCV can be obtained by calling 800-327-2842. Anyone currently receiving Wyeth vaccine should complete the course with Merieux vaccine and does not require an additional booster. Serologic testing is recommended if a systemic allergic reaction (serum sickness or urticaria) occurred during previous administration of postexposure prophylaxis. In that case, an acceptable serologic response obviates the need for a booster vaccine dose. Serum testing continues to be indicated if a patient who received postexposure prophylaxis with HDCV is immunosuppressed (by diseases or medications) (1). State health departments can be contacted for the addresses of laboratories where serologic testing is available.

Wyeth vaccine administered preexposure and in the recommended 1.0 ml intramuscular doses (three

injections) has been effective in inducing antibodies. Based on currently available information, persons so vaccinated need neither serologic testing nor booster doses of HDCV, except for those select groups previously identified (1). In the event of future exposure to rabies, persons who have received preexposure prophylaxis with either type of HDCV should receive two 1.0-ml intramuscular booster doses of HDCV (one each on days 0 and 3), as is currently recommended (1).

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9. Committee on Immunization, Council of Medical Societies, American College of Physicians. Guide for adult immunizations. 1985:1. *At present, CDC considers a neutralizing antibody titer that produces complete inhibition in the rapid fluorescent focus inhibition test at 1:5 dilution or greater (1:11 or greater by the Reed-Muench method) an acceptable response to immunization (1). The World Health Organization considers 0.5 IU/ml or greater (2) an acceptable response (approximately equivalent to 1:56 by the Reed-Muench method or complete inhibition at the 1:25 dilution).

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and Human Services

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Rabies Vaccine
IMOVAX® RABIES

**WISTAR RABIES VIRUS STRAIN PM-1503-3M
GROWN IN HUMAN DIPLOID CELL CULTURES**

**RABIES
(HDCV)**

℞ only

DESCRIPTION

The Imovax® Rabies Vaccine produced by Sanofi Pasteur SA is a sterile, stable, freeze-dried suspension of rabies virus prepared from strain PM-1503-3M obtained from the Wistar Institute, Philadelphia, PA.

The virus is harvested from infected human diploid cells, MRC-5 strain, concentrated by ultrafiltration and is inactivated by beta-propiolactone. One dose of reconstituted vaccine contains less than 100 mg albumin, less than 150 µg neomycin sulfate and 20 µg of phenol red indicator. This vaccine must only be used intramuscularly and as a single dose vial.

The vaccine contains no preservative or stabilizer. It should be used immediately after reconstitution, and if not administered promptly, discard contents.

The potency of one dose (1.0 mL) Sanofi Imovax Rabies Vaccine is equal to or greater than 2.5 International units of rabies antigen.

CLINICAL PHARMACOLOGY

Pre-exposure Immunization

High titer antibody responses of the Sanofi Pasteur SA Imovax Rabies Vaccine made in human diploid cells have been demonstrated in trials conducted in England[1], Germany[2, 3], France[4] and Belgium.[5] Seroconversion was often obtained with only one dose. With two doses one month apart, 100% of the recipients developed specific antibody and the geometric mean titer of the group was approximately 10 international units. In the US, Sanofi Pasteur SA Imovax Rabies Vaccine resulted in geometric mean titers (GMT) of 12.9 IU/mL at Day 49 and 5.1 IU/mL at Day 90 when three doses were given intramuscularly during the course of one month. The range of antibody responses was 2.8 to 55.0 IU/mL at Day 49 and 1.8 to 12.4 IU at Day 90.[6] The definition of a minimally accepted antibody titer varies among laboratories and is influenced by the type of test conducted. CDC currently specifies a 1:5 titer (complete inhibition) by the rapid fluorescent focus inhibition test (RFFIT) as acceptable. The World Health Organization (WHO) specifies a titer of 0.5 IU.[7]

Postexposure Immunization

Postexposure efficacy of Sanofi Pasteur SA Imovax Rabies Vaccine was successfully proven during clinical experience in Iran[7] in conjunction with antirabies serum. Forty-five persons severely bitten by rabid dogs and wolves received Sanofi vaccine within hours of and up to 14 days after the bites. All individuals were fully protected against rabies.

There have been reports of possible vaccine failure when the vaccine has been administered in the gluteal area. Presumably subcutaneous fat in the gluteal area may interfere with the immunogenicity of human diploid cell rabies vaccine (HDCV).[26, 29] For adults and children, Rabies Vaccine should be administered in the deltoid muscle. (See **DOSAGE AND ADMINISTRATION.**)

INDICATIONS AND USAGE

1. Rationale of treatment

Physicians must evaluate each possible rabies exposure. Local or state public health officials should be consulted if questions arise about the need for prophylaxis.[8]

In the United States and Canada, the following factors should be considered before antirabies treatment is initiated.

Species of biting animal

Carnivorous wild animals (especially skunks, raccoons, foxes, coyotes, and bobcats) and bats are the animals most commonly infected with rabies and have caused most of the indigenous cases of human rabies in the United States since 1960. Unless an animal is tested and shown not to be rabid, postexposure prophylaxis should be initiated upon bite or nonbite exposure to the animals. (See definition in "Type of Exposure" below.) If treatment has been initiated and subsequent testing in a competent laboratory shows the exposing animal is not rabid, treatment can be discontinued.[8]

The likelihood that a domestic dog or cat is infected with rabies varies from region to region; hence the need for postexposure prophylaxis also varies.[8]

Rodents (such as squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, and mice) and lagomorphs (including rabbits and hares) are rarely found to be infected with rabies and have not been known to cause human rabies in the United States. In these cases, the state or local health department should be consulted before a decision is made to initiate postexposure antirabies prophylaxis.[8]

Circumstances of biting incident

An UNPROVOKED attack is more likely than a provoked attack to indicate the animal is rabid. Bites inflicted on a person attempting to feed or handle an apparently healthy animal should generally be regarded as PROVOKED.

Type of exposure

Rabies is transmitted by introducing the virus into open cuts or wounds in skin or via mucous membranes. The likelihood of rabies infection varies with the nature and extent of exposure. Two categories of exposure should be considered.

Bite: Any penetration of the skin by teeth.

Nonbite: Scratches, abrasions, open wounds, or mucous membranes contaminated with saliva or other potentially infectious material, such as brain tissue, from a rabid animal. Casual contact, such as petting a rabid animal (without a bite or nonbite exposure as described above), does not constitute an exposure and is not an indication for prophylaxis. There have been two instances of airborne rabies acquired in laboratories and two probable airborne rabies cases acquired in a bat-infested cave in Texas.[8, 9]

The only documented cases for rabies from human-to-human transmission occurred in four patients in the United States and overseas who received corneas transplanted from persons who died of rabies undiagnosed at the time of death.[9, 10] Stringent guidelines for acceptance of donor corneas should reduce this risk. Bite and nonbite exposure from humans with rabies theoretically could transmit rabies, although no cases of rabies acquired this way have been documented. Each potential exposure to human rabies should be carefully evaluated to minimize unnecessary rabies prophylaxis.[8, 11]

2. Pre- and postexposure treatment of rabies

A. Pre-exposure - See TABLE 1

Pre-exposure immunization may be offered to persons in high-risk groups, such as veterinarians, animal handlers, certain laboratory workers, and persons spending time (eg, 1 month or more) in foreign countries where rabies is a constant threat. Persons whose vocational or avocational pursuits bring them into contact with potentially rabid dogs, cats, foxes, skunks, bats, or other species at risk of having rabies should also be considered for pre-exposure prophylaxis.[8]

Vaccination is recommended for children living in or visiting countries where exposure to rabid animals is a constant threat. Worldwide statistics indicate children are more at risk than adults.

Pre-exposure prophylaxis is given for several reasons. First, it may provide protection to persons with inapparent exposure to rabies. Secondly, it may protect persons whose postexposure therapy might be expected to be delayed. Finally, although it does not eliminate the need for additional therapy after a rabies exposure, it simplifies therapy by eliminating the need for globulin and decreasing the number of doses of vaccine needed. This is of particular importance for persons at high risk of being exposed in countries where the available rabies immunizing products may carry a higher risk of adverse reactions.

Pre-exposure immunization does not eliminate the need for prompt prophylaxis following an exposure. It only reduces the postexposure treatment regimen.[8]

PRE-EXPOSURE RABIES TREATMENT GUIDE

1. Pre-exposure immunization: Consists of the three doses of HDCV, 1.0 mL, intramuscularly (deltoid area), one each on Days 0, 7 and 21 or 28. Administration of routine booster doses of vaccine depends on exposure risk category as noted in Table 1. Pre-exposure immunization of immunosuppressed persons is not recommended.[8]

TABLE 1[8]

CRITERIA FOR PRE-EXPOSURE IMMUNIZATION

Risk category	Nature of risk	Typical populations	Pre-exposure regimen
Continuous	Virus present continuously often in high concentrations. Aerosol, mucous membrane, bite or nonbite exposure possible. Specific exposures may go unrecognized.	Rabies research lab workers.* Rabies biologics production workers.	Primary pre-exposure immunization course. Serology every 6 months. Booster immunization when antibody titer falls below acceptable level.*
Frequent	Exposure usually episodic, with source recognized, but exposure may also be unrecognized. Aerosol, mucous membrane, bite or nonbite exposure.	Rabies diagnostic lab workers*, spelunkers, veterinarians, and animal control and wildlife workers in rabies epizootic areas.	Primary pre-exposure immunization course. Booster immunization or serology every 2 years.†
Infrequent (greater than population-at-large)	Exposure nearly always episodic with source recognized. Mucous membrane, bite or nonbite exposure.	Veterinarians and animal control and wildlife workers in areas of low rabies endemicity. Certain travelers to foreign rabies epizootic areas. Veterinary students.	Primary pre-exposure immunization course. No routine booster immunization or serology.
Rare (population-at-large)	Exposure always episodic, mucous membrane, or bite with source recognized.	US population-at-large, including individuals in rabies epizootic areas.	No pre-exposure immunization.

* Judgement of relative risk and extra monitoring of immunization status of laboratory workers is the responsibility of the laboratory supervisor (see US Department of Health and Human Service's Biosafety in Microbiological and Biomedical Laboratories, 1984).

† Pre-exposure booster immunization consists of one dose of HDCV, 1.0 mL/dose, IM (deltoid area). Acceptable antibody level is 1:5 titer (complete inhibition in RFFIT at 1:5 dilution). (See **CLINICAL PHARMACOLOGY.**) Boost if titer falls below 1:5.

B. Postexposure - See TABLE 2

The essential components of rabies postexposure prophylaxis are local treatment of wounds and immunization, including administration, in most instances, of both globulin and vaccine (TABLE 2).[8, 13]

1. Local treatment of wounds: Immediate and thorough washing of all bite wounds and scratches with soap and water is perhaps the most effective measure for preventing rabies. In experimental animals, simple local wound cleansing has been shown to reduce markedly the likelihood of rabies.[8, 11]

Tetanus prophylaxis and measures to control bacterial infection should be given as indicated.

2. Specific treatment: Postexposure antirabies immunization should always include administration of both antibody (preferably RIG) and vaccine, with one exception: persons who have been previously immunized with the recommended pre-exposure or postexposure regimens with HDCV or who have been immunized with other types of vaccines and have a history of documented adequate rabies antibody titer should receive only vaccine. The combination of globulin and vaccine is recommended for both bite exposures and nonbite exposures regardless of the interval between exposure and treatment.[14, 15] The sooner treatment is begun after exposure, the better. However, there have been instances in which the decision to begin treatment was made as late as 6 months or longer after the exposure due to delay in recognition that an exposure had occurred.[8, 13]

3. Treatment outside the United States: If postexposure is begun outside the United States with locally produced biologics, it may be desirable to provide additional treatment when the patient reaches the US. State health departments should be contacted for specific advice in such cases.[8]

POSTEXPOSURE TREATMENT GUIDE

The following recommendations are only a guide. In applying them, take into account the animal species involved, the circumstances of the bite or other exposure, the vaccination status of the animal, and presence of rabies in the region. Local or state public health officials should be consulted if questions arise about the need for rabies prophylaxis.[8]

TABLE 2[8]

Animal species	Condition of animal at time of attack	Treatment of exposed person*
DOMESTIC: Dog and cat	Healthy and available for 10 days of observation. Rabid or suspected rabid. Unknown (escaped).	None unless animal develops rabies.† RIG§ and HDCV. Consult public health officials. If treatment is indicated, give RIG§ and HDCV.
WILD: Skunk, bat, fox, coyote, raccoon, bobcat and other carnivores	Regard as rabid unless proven negative by laboratory tests.£	RIG§ and HDCV.
OTHER: Livestock, rodents and lagomorphs (rabbits and hares)	Consider individually. Local and state public health officials should be consulted on questions about the need for rabies prophylaxis. Bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other rodents, rabbits and hares, almost never call for antirabies prophylaxis.	

* All bites and wounds should immediately be thoroughly cleansed with soap and water. If antirabies treatment is indicated, both rabies immune globulin (RIG) and human diploid cell rabies vaccine (HDCV) should be given as soon as possible regardless of the interval from exposure. Local reactions to vaccines are common and do not contraindicate continuing treatment. Discontinue vaccine if fluorescent antibody tests of the animal are negative.

† During the usual holding period of 10 days, begin treatment with RIG and HDCV at first sign of rabies in a dog or cat that has bitten someone. The symptomatic animal should be killed immediately and tested.

§ If RIG is not available, use antirabies serum, equine (ARS). Do not use more than the recommended dosage.

£ The animal should be killed and tested as soon as possible. Holding for observation is not recommended.

CONTRAINDICATIONS

For postexposure treatment, there are no known specific contraindications to the use of Sanofi Imovax Rabies Vaccine. In cases of pre-exposure immunization, there are no known specific contraindications other than situations such as developing febrile illness, etc.

WARNINGS

Rabies Vaccine in this package is a unit dose to be delivered intramuscularly in the deltoid area.[8]

This vaccine must not be used intradermally or as a multiple dose dispensing unit. In both pre-exposure and postexposure immunization, the full 1.0 mL dose should be given intramuscularly.

In the case of pre-exposure immunization, recently a significant increase has been noted in "immune complex-like" reactions in persons receiving booster doses of HDCV.[16] The illness characterized by onset 2-21 days post-booster, presents with a generalized urticaria and may also include arthralgia, arthritis, angioedema, nausea, vomiting, fever, and malaise. In no cases were the illnesses life-threatening. Preliminary data suggest this "immune complex-like" illness may occur in up to 6% of persons receiving booster vaccines and much less frequently in persons receiving primary immunization. Additional experience with this vaccine is needed to define more clearly the risk of these adverse reactions.[8, 17]

Two cases of neurologic illness resembling Guillain-Barré syndrome[18, 19] a transient neuroparalytic illness, that resolved without sequelae in 12 weeks and a focal subacute central nervous system disorder temporally associated with HDCV, have been reported.[20]

All serious systemic neuroparalytic or anaphylactic reactions to a rabies vaccine should be immediately reported to the state health department or Sanofi Pasteur Inc., 1-800-VACCINE (1-800-822-2463).[8]

PRECAUTIONS

IN ADULTS AND CHILDREN THE VACCINE SHOULD BE INJECTED INTO THE DELTOID MUSCLE. IN INFANTS AND SMALL CHILDREN THE MID-LATERAL ASPECT OF THE THIGH MAY BE PREFERABLE.

General

When a person with a history of hypersensitivity must be given rabies vaccine, antihistamines may be given; epinephrine (1:1000) should be readily available to counteract anaphylactic reactions, and the person should be carefully observed after immunization.

While the concentration of antibiotics in each dose of vaccine is extremely small, persons with known hypersensitivity to any of these agents could manifest an allergic reaction. While the risk is small, it should be weighed in light of the potential risk of contracting rabies.

Drug interactions

Corticosteroids, other immunosuppressive agents, and immunosuppressive illnesses can interfere with the development of active immunity and predispose the patient to developing rabies. Immunosuppressive agents should not be administered during postexposure therapy, unless essential for the treatment of other conditions. When rabies postexposure prophylaxis is administered to persons receiving steroids or other immunosuppressive therapy, it is especially important that serum be tested for rabies antibody to ensure that an adequate response has developed.[8]

Usage in pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with Imovax Rabies Vaccine. It is also not known whether the product can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Rabies vaccine should be given to a pregnant woman only if clearly needed.

Because of the potential consequences of inadequately treated rabies exposure and limited data that indicate that fetal abnormalities have not been associated with rabies vaccination, pregnancy is not considered a contraindication to postexposure prophylaxis.[8, 21] If there is substantial risk of exposure to rabies, pre-exposure prophylaxis may also be indicated during pregnancy.[8]

Pediatric use

Both safety and efficacy in children have been established.

ADVERSE REACTIONS

ALSO SEE WARNINGS AND CONTRAINDICATIONS SECTIONS FOR ADDITIONAL STATEMENTS.

Once initiated, rabies prophylaxis should not be interrupted or discontinued because of local or mild systemic adverse reactions to rabies vaccine. Usually such reactions can be successfully managed with anti-inflammatory and antipyretic agents (eg, aspirin).

Reactions after vaccination with HDCV are less common than with previously available vaccines.[12, 16, 17] In a study using five doses of HDCV, local reactions, such as pain, erythema, and swelling or itching at the injection site were reported in about 25% of recipients of HDCV, and mild systemic reactions such as headache, nausea, abdominal pain, muscle aches and dizziness were reported in about 20% of recipients.[8]

Serious systemic anaphylactic or neuroparalytic reactions occurring during the administration of rabies vaccines pose a dilemma for the attending physician. A patient's risk of developing rabies must be carefully considered before deciding to discontinue vaccination. Moreover, the use of corticosteroids to treat life-threatening neuroparalytic reactions carries the risk of inhibiting the development of active immunity to rabies. It is especially important in these cases that the serum of the patient be tested for rabies antibodies. Advice and assistance on the management of serious adverse reactions in persons receiving rabies vaccines may be sought from the state health department.[8]

DOSAGE AND ADMINISTRATION

Before administration, parenteral drug products should be checked visually for any deviation from normal appearance including container integrity. The syringe and its package should also be inspected prior to use for evidence of leakage, premature activation of the plunger, or a faulty tip seal. If evidence of such defects are observed, the syringe should not be used.

The package contains a vial of freeze-dried vaccine, a syringe containing 1.0 mL of diluent, a plunger for the syringe, and a needle for reconstitution. Attach the plunger and reconstitution needle to the syringe and reconstitute the freeze-dried vaccine by injecting the diluent into the vaccine vial. Gently swirl the contents until completely dissolved and withdraw the total contents of the vial into the syringe. Remove the reconstitution needle and discard. For administration, use a needle of your choice that is suitable for intramuscular injection of your patient.

The syringe is intended for single use only, must not be reused, and must be disposed of properly and promptly following its use.

To help avoid HIV (AIDS), HBV, (Hepatitis), and other infectious diseases due to accidental needlesticks, contaminated needles should not be recapped or removed, unless there is no alternative or that such action is required by a specific medical procedure.

The reconstituted vaccine should be used immediately.

After preparation of the injection site, immediately inject the vaccine intramuscularly. For adults and children, the vaccine should be injected into the deltoid muscle.[22-27,29] In infants and small children, the mid lateral aspect of the thigh may be preferable. Care should be taken to avoid injection into or near blood vessels and nerves. If blood or any suspicious discoloration appears in the syringe, do not inject but discard contents and repeat procedure using a new dose of vaccine, at a different site.

NOTE: The freeze-dried vaccine is creamy white to orange. After reconstitution it is pink to red.

A. Pre-exposure dosage

1. Primary vaccination: In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends three injections of 1.0 mL each, one injection on Day 0 and one on Day 7 and one either on Day 21 or 28.[8]

2. Booster dose: Persons working with live rabies virus in research laboratories and in vaccine production facilities should have rabies antibody titers checked every six months and boosters given as needed to maintain an adequate titer. (For definition of adequate titer, see **CLINICAL PHARMACOLOGY.**) Only laboratory workers, such as those doing rabies diagnostic tests, spelunkers and veterinarians, animal control and wildlife officers in areas where rabies is epizootic should have boosters every 2 years or have their serum tested for rabies antibody every 2 years and, if the titer is inadequate, have a booster dose. Veterinarians and animal control and wildlife officers, if working in areas of low rabies endemicity, do not require routine booster doses of HDCV after completion of primary pre-exposure immunization (TABLE 1.)[8]

Persons who have experienced "immune complex-like" hypersensitivity reactions should receive no further doses of HDCV unless they are exposed to rabies or they are truly likely to be inapparently and/or unavoidably exposed to rabies virus and have unsatisfactory antibody titers.

B. Postexposure dosage

The World Health Organization established a recommendation for six intramuscular doses of human diploid cell vaccine (HDCV) based on studies in Germany and Iran.[3,7] Used in this way, a total of 6 injections of a 1.0 mL dose of vaccine are given according to the following schedule: on Day 0, 3, 7, 14, 30 and 90. The first dose should be accompanied by Rabies Immune Globulin (RIG) or Antirabies Serum (ARS). If possible, up to half the dose of RIG or ARS should be used to infiltrate the wound, and the rest administered intramuscularly, in a different site from the rabies vaccine, preferably in the gluteal region.

Studies conducted at the CDC in the United States have shown that a regimen of 1 dose of Rabies Immune Globulin (RIG) and 5 doses of HDCV induced an excellent antibody response in all recipients. Of 511 persons bitten by proven rabid animals and so treated, none developed rabies.[8]

Based on these data, the ACIP recommends a 5-dose regimen for postexposure situations. Five 1.0 mL doses are given intramuscularly on Day 0, 3, 7, 14 and 28 in conjunction with RIG on Day 0.[8]

Because the antibody response following the recommended vaccination regimen with HDCV has been so satisfactory, routine postvaccination serologic testing is not recommended. Serologic testing is indicated in unusual circumstances, as when the patient is known to be immunosuppressed. Contact state health department or CDC for recommendations.[8,28]

C. Postexposure therapy of previously immunized persons

When an immunized person who was vaccinated by the recommended regimen with a cell culture vaccine or who had previously demonstrated rabies antibody is exposed to rabies, that person should receive two intramuscular doses (1.0 mL each) of HDCV, one immediately and one 3 days later. RIG should not be given in these cases. If the immune status of a previously vaccinated person who did not receive the recommended HDCV regimen is not known, full primary postexposure antirabies treatment (RIG plus 5 doses of HDCV) may be necessary. In such cases, if antibody can be demonstrated in a serum sample collected before vaccine is given, treatment can be discontinued after at least two doses of HDCV.[8]

HOW SUPPLIED

IMOVAX RABIES VACCINE is supplied in a tamper evident unit dose box with:

- One vial of freeze-dried vaccine containing a single dose.
- One syringe containing diluent. A separate plunger is provided for insertion and use.
- One disposable needle for reconstitution.

Product No. 49281-250-51

"CPT ® Code: 90675

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STORAGE

The freeze-dried vaccine is stable if stored in the refrigerator at 2°C to 8°C (35°F to 46°F). Do not freeze.

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Product information as of December 2005

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Manufactured by:
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sanofi pasteur

RABAVERT - rabies virus inactivated antigen, a injection, powder, lyophilized, for suspension
Novartis Vaccines and Diagnostics GmbH & Co. KG

Rabies Vaccine for Human Use

DESCRIPTION

RabAvert, Rabies Vaccine, produced by Novartis Vaccines and Diagnostics GmbH & Co. KG is a sterile freeze-dried vaccine obtained by growing the fixed-virus strain Flury LEP in primary cultures of chicken fibroblasts. The strain Flury LEP was obtained from American Type Culture Collection as the 59th egg passage. The growth medium for propagation of the virus is a synthetic cell culture medium with the addition of human albumin, polygeline (processed bovine gelatin) and antibiotics. The virus is inactivated with β-propiolactone, and further processed by zonal centrifugation in a sucrose density-gradient. The vaccine is lyophilized after addition of a stabilizer solution which consists of buffered polygeline and potassium glutamate. One dose of reconstituted vaccine contains less than 12 mg polygeline (processed bovine gelatin), less than 0.3 mg human serum albumin, 1 mg potassium glutamate and 0.3 mg sodium EDTA. Small quantities of bovine serum are used in the cell culture process. Bovine components originate only from the United States, Australia and New Zealand. Minimal amounts of chicken protein may be present in the final product; ovalbumin content is less than 3 ng/dose (1 mL), based on ELISA. Antibiotics (neomycin, chlortetracycline, amphotericin B) added during cell and virus propagation are largely removed during subsequent steps in the manufacturing process. In the final vaccine, neomycin is present at < 1 µg, chlortetracycline at < 20 ng, and amphotericin B at < 2 ng per dose. RabAvert is intended for intramuscular (IM) injection. The vaccine contains no preservative and should be used immediately after reconstitution with the supplied Sterile Diluent for RabAvert (Water For Injection). The potency of the final product is determined by the NIH mouse potency test using the US reference standard. The potency of one dose (1.0 mL) RabAvert is at least 2.5 IU of rabies antigen. RabAvert is a white, freeze-dried vaccine for reconstitution with the diluent prior to use; the reconstituted vaccine is a clear to slightly opaque, colorless suspension.

CLINICAL PHARMACOLOGY

Rabies in the United States

Over the last 100 years, the epidemiology of rabies in animals in the United States has changed dramatically. More than 90% of all animal rabies cases reported annually to the Centers for Disease Control and Prevention (CDC) now occur in wildlife, whereas before 1960 the majority were in domestic animals. The principal rabies hosts today are wild terrestrial carnivores and bats. Annual human deaths have fallen from more than a hundred at the turn of the century to one to two per year despite major epizootics of animal rabies in several geographic areas. Within the United States, only Hawaii has remained rabies free. Although rabies among humans is rare in the United States, every year tens of thousands of people receive rabies vaccine for postexposure prophylaxis.

Rabies is a viral infection transmitted via the saliva of infected mammals. The virus enters the central nervous system of the host, causing an encephalomyelitis that is almost invariably fatal. The incubation period varies between 5 days and several years, but is usually between 20 and 60 days. Clinical rabies presents either in a furious or in a paralytic form. Clinical illness most often starts with prodromal complaints of malaise, anorexia, fatigue, headache, and fever followed by pain or paresthesia at the site of exposure. Anxiety, agitation, irritability may be prominent during this period, followed by hyperactivity, disorientation, seizures, aero- and hydrophobia, hypersalivation, and eventually paralysis, coma and death.

Modern day prophylaxis has proven nearly 100% successful; most human fatalities now occur in people who fail to seek medical treatment, usually because they do not recognize a risk in the animal contact leading to the infection. Inappropriate postexposure prophylaxis may also result in clinical rabies. Survival after clinical rabies is extremely rare, and is associated with severe brain damage and permanent disability.

RabAvert (in combination with passive immunization with Human Rabies Immune Globulin [HRIG] and local wound treatment) in postexposure treatment against rabies has been shown to protect patients of all age groups from rabies, when the vaccine was administered according to the CDC's Advisory Committee on Immunization Practices (ACIP) or World Health Organization (WHO) guidelines and as soon as possible after rabid animal contact. Anti-rabies antibody titers after immunization have been shown to reach levels well above the minimum antibody titer accepted as seroconversion (protective titer) within 14 days after initiating the postexposure treatment series. The minimum antibody titer accepted as seroconversion is a 1:5 titer (complete inhibition in the rapid fluorescent focus inhibition test [RFFIT] at 1:5 dilution) as specified by the CDC (1), or ≥ 0.5 IU per milliliter (mL) as specified by the WHO (2,3).

Clinical Studies

Preexposure Vaccination

The immunogenicity of RabAvert has been demonstrated in clinical trials conducted in different countries such as the USA (4,5), UK (6), Croatia (7), and Thailand (8-10). When administered according to the recommended immunization schedule (days 0, 7, 21 or 0, 7, 28), 100% of subjects attained a protective titer. In two studies carried out in the USA in 101 subjects, antibody titers > 0.5 IU/mL were obtained by day 28 in all subjects. In studies carried out in Thailand in 22 subjects, and in Croatia in 25 subjects, antibody titers of > 0.5 IU/mL were obtained by day 14 (injections on days 0, 7, 21) in all subjects.

The ability of RabAvert to boost previously immunized subjects was evaluated in three clinical trials. In the Thailand study, preexposure booster doses were administered to 10 individuals. Antibody titers of > 0.5 IU/mL were present at baseline on day 0 in all subjects (9). Titers after a booster dose were enhanced from geometric mean titers (GMT) of 1.91 IU/mL to 23.66 IU/mL on day 30. In an additional booster study, individuals known to have been immunized with Human Diploid Cell Vaccine (HDCV) were boosted with RabAvert. In this study, a booster response was observed on day 14 for all (22/22) individuals (11). In a trial carried out in the USA (4), a RabAvert IM booster dose resulted in a significant increase in titers in all (35/35) subjects, regardless of whether they had received RabAvert or HDCV as the primary vaccine.

Persistence of antibody after immunization with RabAvert has been evaluated. In a trial performed in the UK, neutralizing antibody titers > 0.5 IU/mL were present 2 years after immunization in all sera (6/6) tested.

Preexposure Vaccination in Children
Preexposure administration of RabAvert in 11 Thai children from the age of 2 years and older resulted in antibody levels higher than 0.5 IU/mL on day 14 in all children (12).

Postexposure Treatment
RabAvert, when used in the recommended postexposure WHO program of 5 to 6 IM injections of 1 mL (days 0, 3, 7, 14, 30, and one optionally on day 90) provided protective titers of neutralizing antibody (> 0.5 IU/mL) in 158/160 patients (8, 9, 13-16) within 14 days and in 215/216 patients by day 28 - 38.

Of these, 203 were followed for at least 10 months. No case of rabies was observed (8, 9, 13-20). Some patients received Human Rabies Immune Globulin (HRIG), 20 - 30 IU per kg body weight, or Equine Rabies Immune Globulin (ERIG), 40 IU per kg body weight, at the time of the first dose. In most studies (8, 9, 13, 17), the addition of either HRIG or ERIG caused a slight decrease in GMTs which was neither clinically relevant nor statistically significant. In one study (16), patients receiving HRIG had significantly lower ($p < 0.05$) GMTs on day 14; however, again this was not clinically relevant. After day 14 there was no statistical significance.

The results of several studies of normal volunteers receiving the postexposure WHO regimen, i.e., "simulated" postexposure, show that with sampling by day 28 - 30, 205/208 vaccinees had protective titers > 0.5 IU/mL.

No postexposure vaccine failures have occurred in the United States since cell culture vaccines have been routinely used (1). Failures have occurred abroad, almost always after deviation from the recommended postexposure treatment protocol (21-24). In two cases with bites to the face, treatment failed although no deviation from the recommended postexposure treatment protocol appeared to have occurred (25).

Postexposure Treatment in Children
In a 10-year serosurveillance study, RabAvert has been administered to 91 children aged 1 to 5 years and 436 children and adolescents aged 6 to 20 years (19). The vaccine was effective in both age groups. None of these patients developed rabies.

One newborn has received RabAvert on an immunization schedule of days 0, 3, 7, 14 and 30; the antibody concentration on day 37 was 2.34 IU/mL. There were no clinically significant adverse events (26).

INDICATIONS AND USAGE

RabAvert is indicated for preexposure vaccination, in both primary series and booster dose, and for postexposure prophylaxis against rabies in all age groups.
Usually, an immunization series is initiated and completed with one vaccine product. No clinical studies have been conducted that document a change in efficacy or the frequency of adverse reactions when the series is completed with a second vaccine product. However, for booster immunization, RabAvert was shown to elicit protective antibody level responses in persons tested who received a primary series with HDCV (4,11).

A. Preexposure Vaccination - See Table 1
(see also Dosage and Administration section below)
Preexposure vaccination consists of three doses of RabAvert 1.0 mL, intramuscularly (deltoid region), one each on days 0, 7, and 21 or 28 (1) (see also Table 1 for criteria for preexposure vaccination).
Preexposure vaccination does not eliminate the need for additional therapy after a known rabies exposure (see also Dosage and Administration section, subsection C).
Preexposure vaccination should be offered to persons in high-risk groups, such as veterinarians, animal handlers, wildlife officers in areas where animal rabies is enzootic, certain laboratory workers, and persons spending time in foreign countries where rabies is endemic. Persons whose activities bring them into contact with potentially rabid dogs, cats, foxes, skunks, bats, or other species at risk of having rabies should also be considered for preexposure vaccination. International travelers might be candidates for preexposure vaccination if they are likely to come in contact with animals in areas where dog rabies is enzootic and immediate access to appropriate medical care, including biologics, might be limited (27, 28)

Preexposure vaccination is given for several reasons. First, it may provide protection to persons with inapparent exposure to rabies. Second, it may protect persons whose postexposure therapy might be expected to be delayed. Finally, although it does not eliminate the need for prompt therapy after a rabies exposure, it simplifies therapy by eliminating the need for globulin and decreasing the number of doses of vaccine needed. This is of particular importance for persons at high risk of being exposed in countries where the available rabies immunizing products may carry a higher risk of adverse reactions.
In some instances, booster doses of vaccine should be administered to maintain a serum titer corresponding to at least complete neutralization at a 1:5 serum dilution by the RFFIT (see Table 1); each booster immunization consists of a single dose. See **Clinical Pharmacology**. Serum antibody determinations to decide upon the need for a booster dose is suggested by the ACIP and is considered cost-effective.

Table 1: Rabies Preexposure prophylaxis guide – United States, 1999

Risk Category and Nature of Risk	Typical Populations	Preexposure Recommendations
Continuous. Virus present continuously, often in high concentrations. Specific exposures likely to go unrecognized. Bite, nonbite or aerosol exposure.	Rabies research lab workers,* rabies biologics production workers.	Primary course. Serologic testing every 6 months; booster vaccination if antibody titer is below acceptable level.*
Frequent. Exposure usually episodic, with source recognized, but exposure might be unrecognized. Bite, nonbite or aerosol exposure.	Rabies diagnostic lab workers,* spelunkers, veterinarians and staff, and animal-control and wildlife workers in rabies enzootic areas.	Primary course. Serologic testing every 2 years; booster vaccination if antibody titer is below acceptable level.**
Infrequent (greater than population-at-large). Exposure nearly always episodic with source recognized. Bite or nonbite exposure.	Veterinarians and animal- control and wildlife workers in areas with low rabies rates. Veterinary students. Travelers visiting areas where rabies in enzootic and immediate access to appropriate medical care including biologics is limited.	Primary course. No serologic testing or booster vaccination.**
Rare (population-at-large). Exposures always episodic. with source recognized. Bite or nonbite exposure.	US population-at-large, including persons in rabies-epizootic areas.	No vaccination necessary.

Adapted from the Recommendations of the Advisory Committee on Immunization Practices: Human Rabies Prevention – United States, 1999. (1)

* Judgment of relative risk and extra monitoring of vaccination status of laboratory workers is the responsibility of the laboratory supervisor (29).

** Minimum acceptable antibody level is complete virus neutralization at a 1:5 serum dilution by RFFIT. A booster dose should be administered if the titer falls below this level.

B. Postexposure Treatment - See Table 2
(see also **Dosage and Administration** section below)
The following recommendations are only a guide. In applying them, take into account the animal species involved, the circumstances of the bite or other exposure, the immunization status of the animal, and presence of rabies in the region (as outlined below). Local or state public health officials should be consulted if questions arise about the need for rabies prophylaxis (1).

TABLE 2: RABIES POSTEXPOSURE PROPHYLAXIS GUIDE – UNITED STATES, 1999

Animal type	Evaluation and disposition of animal	Postexposure prophylaxis recommendations
Dogs, cats and ferrets	Healthy and available for 10 days observation Rabid or suspected rabid Unknown (e.g., escaped)	Should not begin prophylaxis unless animal develops clinical signs of rabies* Immediately vaccinate Consult public health officials
Skunks, raccoons, bats, foxes, and most other carnivores	Regarded as rabid unless animal proven negative by laboratory tests**	Consider immediate vaccination
Livestock, small rodents, lagomorphs (rabbits and hares), large rodents (woodchucks and beavers), and other mammals	Consider individually	Consult public health officials. Bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other small rodents, rabbits, and hares almost never require antirabies postexposure prophylaxis

Adapted from the Recommendations of the Advisory Committee on Immunization Practices: Human Rabies Prevention – United States, 1999. (1)

* During the 10-day observation period, begin postexposure prophylaxis at the first sign of rabies in a dog, cat or ferret that has bitten someone. If the animal exhibits clinical signs of rabies, it should be euthanized immediately and tested.

** The animal should be euthanized and tested as soon as possible. Holding for observation is not recommended. Discontinue vaccine if immunofluorescence test results of the animal are negative.

In the United States, the following factors should be considered before antirabies treatment is initiated.

Species of Biting Animal

Wild terrestrial animals (especially skunks, raccoons, foxes and coyotes) and bats are the animals most commonly infected with rabies and are the most important potential source of infection for both humans and domestic animals. Unless a wild animal is tested and shown not to be rabid, postexposure prophylaxis should be initiated upon bite or nonbite exposure to the animals (see definition in "Type of Exposure" below). If treatment has been initiated and subsequent testing in a qualified laboratory shows the exposing animal is not rabid, postexposure prophylaxis can be discontinued (1).

The likelihood of rabies in a domestic animal varies from region to region; hence the need for postexposure prophylaxis also varies (1).

Small rodents (such as squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, and mice) and lagomorphs (including rabbits and hares) are almost never found to be infected with rabies and have not been known to transmit rabies to humans in the United States. Bites from large rodents such as woodchucks (including groundhogs) and beavers, should be considered as possible rabies exposures, especially in regions where rabies is enzootic in raccoons (30). In all cases involving rodents, the state or local health department should be consulted before a decision is made to initiate antirabies postexposure prophylaxis (1).

Circumstances of Biting Incident

An UNPROVOKED attack is more likely than a provoked attack to indicate the animal is rabid. Bites inflicted on a person attempting to feed or handle an apparently healthy animal should generally be regarded as PROVOKED. A currently vaccinated dog, cat or ferret is unlikely to become infected with rabies (1).

Type of Exposure

Rabies is transmitted by introducing the virus into open cuts or wounds in skin or via mucous membranes. The likelihood of rabies infection varies with the nature and extent of exposure. Two categories of exposure should be considered:

Bite: Any penetration of the skin by teeth. Bites to highly innervated areas such as the face and hands carry the highest risk, but the site of the bite should not influence the decision to begin treatment. Recent epidemiologic data suggest that even the very limited injury inflicted by a bat bite (compared to lesions caused by terrestrial carnivores) should prompt consideration of postexposure prophylaxis unless the bat is available for testing and is negative for evidence of rabies (1).

Nonbite: The contamination of open wounds, abrasions, mucous membranes, or theoretically, scratches, with saliva or other potentially infectious material (such as neural tissue) from a rabid animal constitutes a nonbite exposure. In all instances of potential human exposures involving bats, and the bat is not available for testing, postexposure prophylaxis might be appropriate even if a bite, scratch or mucous membrane exposure is not apparent when there is reasonable probability that such exposure might have occurred. Postexposure prophylaxis can be considered for persons who were in the same room as the bat and who might be unaware that a bite or direct contact had occurred (e.g., a sleeping person awakens to find a bat in the room or an adult witnesses a bat in the room with a previously unattended child, mentally disabled person, or intoxicated person) and rabies cannot be ruled out by testing the bat. Other contact by itself, such as petting a rabid animal and contact with blood, urine, or feces (e.g., guano) of a rabid animal, does not constitute an exposure and is not an indication for prophylaxis. Because the rabies virus is inactivated by desiccation and ultraviolet irradiation, in general, if the material containing the virus is dry, the virus can be considered noninfectious. Two cases of rabies have been attributed to probable aerosol exposures in laboratories, and two cases of rabies in Texas could possibly have been due to airborne exposures in caves containing millions of bats (1).

The only documented cases for rabies from human-to-human transmission occurred in eight patients, including two in the USA, who received corneas transplanted from persons who died of rabies undiagnosed at the time of death (1). Stringent guidelines for acceptance of donor corneas have been implemented to reduce this risk.

Bite and nonbite exposure from humans with rabies theoretically could transmit rabies, but no laboratory-diagnosed cases occurring under such situations have been documented. Each potential exposure to human rabies should be carefully evaluated to minimize unnecessary rabies prophylaxis (1).

Postexposure Treatment Schedule

(see also **Dosage and Administration** section below)

The essential components of rabies postexposure prophylaxis are prompt local treatment of wounds and administration of both Human Rabies Immune Globulin (HRIG) and vaccine.

A complete course of postexposure treatment for previously unvaccinated adults and children consists of a total of 5 doses of vaccine, each 1.0 mL: one IM injection (deltoid) on each of days 0, 3, 7, 14 and 28. For previously immunized adults and children, a total of 2 doses of vaccine, each 1.0 mL: one IM injection (deltoid) on each of days 0 and 3. No HRIG should be administered to previously vaccinated persons as it may blunt their rapid memory response to rabies antigen.

1. Local Treatment of Wounds
Immediate and thorough washing of all bite wounds and scratches with soap and water is an important measure for preventing rabies. In animal studies, thorough local wound cleansing alone has been shown to reduce markedly the likelihood of rabies. Whenever possible, bite injuries should not be sutured to avoid further and/or deeper contamination. Tetanus prophylaxis and measures to control bacterial infection should be given as indicated (1).

2. Postexposure Prophylaxis of Rabies
The regimen for postexposure prophylaxis depends on whether or not the patient has been previously immunized against rabies (see below). For persons who have not previously been immunized against rabies, the schedule consists of an initial injection IM of HRIG exactly 20 IU per kilogram body weight in total. If anatomically feasible, the FULL DOSE of HRIG should be thoroughly infiltrated in the area around and into the wounds. Any remaining volume of HRIG should be injected IM at a site distant from rabies vaccine administration. HRIG should never be administered in the same syringe or in the same anatomical site as the rabies vaccine. HRIG is administered only once (for specific instructions for HRIG use, see the product package insert). The HRIG injection is followed by a series of 5 individual injections of RabAvert (1.0 mL each) given IM on days 0, 3, 7, 14 and 28. Postexposure rabies prophylaxis should begin the same day exposure occurred or as soon after exposure as possible. The combined use of HRIG and RabAvert is recommended by the CDC for both bite and non-bite exposures, regardless of the interval between exposure and initiation of treatment.

In the event that HRIG is not readily available for the initiation of treatment, it can be given through the seventh day after administration of the first dose of vaccine. HRIG is not indicated beyond the seventh day because an antibody response to RabAvert is presumed to have begun by that time (1).

The sooner treatment is begun after exposure, the better. However, there have been instances in which the decision to begin treatment was made as late as 6 months or longer after exposure due to delay in recognition that an exposure had occurred. Postexposure antirabies treatment should always include administration of both passive antibody (HRIG) and immunization, with the exception of persons who have previously received complete immunization regimens (preexposure or postexposure) with a cell culture vaccine, or persons who have been immunized with other types of vaccines and have had documented rabies antibody titers. Persons who have previously received rabies immunization should receive 2 IM doses of RabAvert: 1 on day 0 and another on day 3. They should not be given HRIG as this may blunt their rapid memory response to rabies antigen.

3. Postexposure Prophylaxis Outside the United States
If postexposure treatment is begun outside the United States with regimens or biologics that are not used in the United States, it may be prudent to provide additional treatment when the patient reaches the USA. State or local health departments should be contacted for specific advice in such cases (1).

CONTRAINDICATIONS
In view of the almost invariably fatal outcome of rabies, there is no contraindication to postexposure prophylaxis, including pregnancy (1).

Hypersensitivity
History of anaphylaxis to the vaccine or any of the vaccine components constitutes a contraindication to preexposure vaccination with this vaccine.
In the case of postexposure prophylaxis, if an alternative product is not available, the patient should be vaccinated with caution with the necessary medical equipment and emergency supplies available and observed carefully after vaccination. A patient's risk of acquiring rabies must be carefully considered before deciding to discontinue vaccination. Advice and assistance on the management of serious adverse reactions for persons receiving rabies vaccines may be sought from the state health department or CDC.

WARNINGS
Anaphylaxis, encephalitis including death, meningitis, neuroparalytic events such as encephalitis, transient paralysis, Guillain-Barre Syndrome, myelitis, and retrobulbar neuritis; and multiple sclerosis have been reported to be temporally associated with the use of RabAvert. See **Precautions** and **Adverse Events** sections. A patient's risk of developing rabies must be carefully considered, however, before deciding to discontinue immunization.
RABAVERT MUST NOT BE USED SUBCUTANEOUSLY OR INTRADERMALLY.

RabAvert must be injected intramuscularly. For adults, the deltoid area is the preferred site of immunization; for small children and infants, administration into the anterolateral zone of the thigh is preferred. The use of the gluteal region should be avoided, since administration in this area may result in lower neutralizing antibody titers (1).
DO NOT INJECT INTRAVASCULARLY.
Unintentional intravascular injection may result in systemic reactions, including shock. Immediate measures include catecholamines, volume replacement, high doses of corticosteroids, and oxygen.
Development of active immunity after vaccination may be impaired in immune-compromised individuals. Please refer to **Drug Interactions**, under **Precautions**.
This product contains albumin, a derivative of human blood. It is present in RabAvert at concentrations of less than 0.3 mg/dose. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeld-Jakob disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

PRECAUTIONS

General
Care is to be taken by the health care provider for the safe and effective use of the product. The health care provider should also question the patient, parent or guardian about 1) the current health status of the vaccinee; and 2) reactions to a previous dose of RabAvert, or a similar product. Preexposure vaccination should be postponed in the case of sick and convalescent persons, and those considered to be in the incubation stage of an infectious disease. A separate, sterile syringe and needle or a sterile disposable unit should be used for each patient to prevent transmission of hepatitis and other infectious agents from person to person. Needles should not be recapped and should be properly disposed of. As with any rabies vaccine, vaccination with RabAvert may not protect 100% of susceptible individuals.

Hypersensitivity
At present there is no evidence that persons are at increased risk if they have egg hypersensitivities that are not anaphylactic or anaphylactoid in nature. Although there is no safety data regarding the use of RabAvert in patients with egg allergies, experience with other vaccines derived from primary cultures of chick embryo fibroblasts demonstrates that documented egg hypersensitivity does not necessarily predict an increased likelihood of adverse reactions. There is no evidence to indicate that persons with allergies to chickens or feathers are at increased risk of reaction to vaccines produced in primary cultures of chick embryo fibroblasts.
Since reconstituted RabAvert contains processed bovine gelatin and trace amounts of chicken protein, neomycin, chlortetracycline and amphotericin B, the possibility of allergic reactions in individuals hypersensitive to these substances should be considered when administering the vaccine.
Epinephrine injection (1:1000) must be immediately available should anaphylactic or other allergic reactions occur.
When a person with a history of hypersensitivity must be given RabAvert, antihistamines may be given; epinephrine (1:1000), volume replacement, corticosteroids and oxygen should be readily available to counteract anaphylactic reactions.

Drug Interactions
Radiation therapy, antimalarials, corticosteroids, other immunosuppressive agents and immunosuppressive illnesses can interfere with the development of active immunity after vaccination, and may diminish the protective efficacy of the vaccine. Preexposure vaccination should be administered to such persons with the awareness that the immune response may be inadequate. Immunosuppressive agents should not be administered during postexposure therapy unless essential for the treatment of other conditions. When rabies postexposure prophylaxis is administered to persons receiving corticosteroids or other immunosuppressive therapy, or who are immunosuppressed, it is important that a serum sample on day 14 (the day of the fourth vaccination) be tested for rabies antibody to ensure that an acceptable antibody response has been induced (1).
HRIG must not be administered at more than the recommended dose, since active immunization to the vaccine may be impaired.
No data are available regarding the concurrent administration of RabAvert with other vaccines.

Carcinogenesis, Mutagenesis, Impairment of Fertility
Long-term studies with RabAvert have not been conducted to assess the potential for carcinogenesis, mutagenesis, or impairment of fertility.

Use in Pregnancy
Pregnancy Category C. Animal reproductive studies have not been conducted with RabAvert. It is also not known whether RabAvert can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. RabAvert should be given to a pregnant woman only if clearly needed. The ACIP has issued recommendations for use of rabies vaccine in pregnant women (1).

Use in Nursing Mothers
It is not known whether RabAvert is excreted in animal or human milk, but many drugs are excreted in human milk. Although there are no data, because of the potential consequences of inadequately treated rabies exposure, nursing is not considered a contraindication

to postexposure prophylaxis. If the risk of exposure to rabies is substantial, preexposure vaccination might also be indicated during nursing.

Pediatric Use
Children and infants receive the same dose of 1 mL, given IM, as do adults.
Only limited data on the safety and efficacy of RabAvert in the pediatric age group are available. However, in three studies some preexposure and postexposure experience has been gained (12, 19, 26; see also **Clinical Studies** in **Clinical Pharmacology** section).

Geriatric Use
Clinical studies of RabAvert did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients.

ADVERSE REACTIONS
In very rare cases, neurological and neuroparalytical events have been reported in temporal association with administration of RabAvert (see also **Warnings** section). These include cases of hypersensitivity (see **Contraindications, Warnings, and Precautions** sections).
The most commonly occurring adverse reactions are injection site reactions, such as injection site erythema, induration and pain; flu-like symptoms, such as asthenia, fatigue, fever, headache, myalgia and malaise; arthralgia, dizziness, lymphadenopathy, nausea, and rash.
A patient's risk of acquiring rabies must be carefully considered before deciding to discontinue vaccination. Advice and assistance on the management of serious adverse reactions for persons receiving rabies vaccines may be sought from the state health department or CDC (see also **Contraindications** section).
Local reactions such as induration, swelling and reddening have been reported more often than systemic reactions. In a comparative trial in normal volunteers, Dreesen *et al.* (4) described their experience with RabAvert compared to a HDCV rabies vaccine. Nineteen subjects received RabAvert and 20 received HDCV. The most commonly reported adverse reaction was pain at the injection site, reported in 45% of the HDCV group, and 34% of the RabAvert group. Localized lymphadenopathy was reported in about 15% of each group. The most common systemic reactions were malaise (15 % RabAvert group vs. 25 % HDCV group), headache (10 % RabAvert group vs. 20 % HDCV group), and dizziness (15 % RabAvert group vs. 10 % HDCV group). In a recent study in the USA (5), 83 subjects received RabAvert and 82 received HDCV. Again, the most common adverse reaction was pain at the injection site in 80% in the HDCV group and 84% in the RabAvert group. The most common systemic reactions were headache (52% RabAvert group vs. 45% HDCV group), myalgia (53% RabAvert group vs. 38% HDCV group) and malaise (20% RabAvert group vs. 17% HDCV group). None of the adverse events were serious, almost all adverse events were of mild or moderate intensity. Statistically significant differences between vaccination groups were not found. Both vaccines were generally well tolerated.
Uncommonly observed adverse events include temperatures above 38°C (100°F), swollen lymph nodes, pain in limbs and gastrointestinal complaints. In rare cases, patients have experienced severe headache, fatigue, circulatory reactions, sweating, chills, monoarthritis and allergic reactions; transient paresthesias and one case of suspected urticaria pigmentosa have also been reported.

Observed During Clinical Practice (See Warnings and Precautions)
The following adverse reactions have been identified during post approval use of RabAvert. Because these reactions are reported voluntarily from a population of uncertain size, estimates of frequency cannot be made. These events have been chosen for inclusion due to their seriousness, frequency of reporting, causal connection to RabAvert, or a combination of these factors:

Allergic: Anaphylaxis, Type III hypersensitivity-like reactions, bronchospasm, urticaria, pruritis, edema

CNS: Neuroparalysis, encephalitis, meningitis, transient paralysis, Guillain-Barre Syndrome, myelitis, retrobulbar neuritis, multiple sclerosis, vertigo, visual disturbance

Cardiac: Palpitations, hot flush

Local: Extensive limb swelling

The use of corticosteroids to treat life-threatening neuroparalytic reactions may inhibit the development of immunity to rabies (see **Precautions, Drug Interactions**).
Once initiated, rabies prophylaxis should not be interrupted or discontinued because of local or mild systemic adverse reactions to rabies vaccine. Usually such reactions can be successfully managed with anti-inflammatory and antipyretic agents.

Reporting of Adverse Events
Adverse events should be reported by the health care provider or patient to the US Department of Health and Human Services (DHHS) Vaccine Adverse Event Reporting System (VAERS). Report forms and information about reporting requirements or completion of the form can be obtained from VAERS by calling the toll-free number 1-800-822-7967 (1). In the USA, such events can be reported to the Professional Services department, Novartis Vaccines and Diagnostics, Inc.: phone: 1-800-244-7668.

DOSAGE AND ADMINISTRATION

The individual dose for adults, children, and infants is 1 mL, given intramuscularly.
In adults, administer vaccine by IM injection into the deltoid muscle. In small children and infants, administer vaccine into the anterolateral zone of the thigh. The gluteal area should be avoided for vaccine injections, since administration in this area may result in lower neutralizing antibody titers. Care should be taken to avoid injection into or near blood vessels and nerves. After aspiration, if blood or any suspicious discoloration appears in the syringe, do not inject but discard contents and repeat procedure using a new dose of vaccine, at a different site.

A. Preexposure Dosage

1. Primary Immunization
In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends three injections of 1.0 mL each: one injection on day 0 and one on day 7, and one either on day 21 or 28 (for criteria for preexposure vaccination, see Table 1).

2. Booster Immunization
The individual booster dose is 1 mL, given intramuscularly.

Booster immunization is given to persons who have received previous rabies immunization and remain at increased risk of rabies exposure by reasons of occupation or avocation.

Persons who work with live rabies virus in research laboratories or vaccine production facilities (continuous-risk category: see Table 1) should have a serum sample tested for rabies antibodies every 6 months. The minimum acceptable antibody level is complete virus neutralization at a 1:5 serum dilution by the rapid fluorescent focus inhibition test (RFFIT). A booster dose should be administered if the titer falls below this level.

The frequent-risk category includes other laboratory workers such as those doing rabies diagnostic testing, spelunkers, veterinarians and staff, animal-control and wildlife officers in areas where rabies is epizootic. Persons in the frequent-risk category should have a serum sample tested for rabies antibodies every 2 years and, if the titer is less than complete neutralization at a 1:5 serum dilution by RFFIT, should have a booster dose of vaccine. Alternatively, a booster can be administered in the absence of a titer determination.

The infrequent-risk category, including veterinarians, animal-control and wildlife officers working in areas of low rabies enzooticity (infrequent-exposure group) and international travelers to rabies enzootic areas do not require routine preexposure booster doses of RabAvert after completion of a full primary preexposure vaccination scheme (Table 1).

B. Postexposure Dosage

Immunization should begin as soon as possible after exposure. A complete course of immunization consists of a total of 5 injections of 1 mL each: one injection on each of days 0, 3, 7, 14 and 28 in conjunction with the administration of HRIG on day 0. For children, see **Pediatric Use** section under **Precautions.**
Begin with the administration of HRIG. Give 20 IU/kg body weight.
This formula is applicable to all age groups, including infants and children. The recommended dosage of HRIG should not exceed 20 IU/kg body weight because it may otherwise interfere with active antibody production. Since vaccine-induced antibody appears within 1 week, HRIG is not indicated more than 7 days after initiating postexposure prophylaxis with RabAvert. If anatomically feasible, the FULL DOSE of HRIG should be thoroughly infiltrated in the area around and into the wounds. Any remaining volume of HRIG should be injected IM at a site distant from rabies vaccine administration. HRIG should never be administered in the same syringe or in the same anatomical site as the rabies vaccine.
Because the antibody response following the recommended immunization regimen with RabAvert has been satisfactory, routine post-immunization serologic testing is not recommended. Serologic testing is indicated in unusual circumstances, as when the patient is known to be immunosuppressed. Contact the appropriate state health department or the CDC for recommendations.

C. Postexposure Prophylaxis of Previously Immunized Persons

When rabies exposure occurs in a previously vaccinated person, then that person should receive two IM (deltoid) doses (1.0 mL each) of RabAvert: one immediately and one 3 days later. HRIG should not be given in these cases. Persons considered to have been immunized previously are those who received a complete preexposure vaccination or postexposure prophylaxis with RabAvert or other tissue culture vaccines or have been documented to have had a protective antibody response to another rabies vaccine. If the immune status of a previously vaccinated person is not known, full postexposure antirabies treatment (HRIG plus 5 doses of vaccine) is recommended. In such cases, if a protective titer can be demonstrated in a serum sample collected before vaccine is given, treatment can be discontinued after at least two doses of vaccine.

Instructions for Reconstituting RabAvert

Using the longer of the 2 needles supplied, withdraw the entire contents of the Sterile Diluent for RabAvert into the syringe. Insert the needle at a 45° angle and slowly inject the entire contents of the diluent vial into the vaccine vial. Mix gently to avoid foaming.

The white, freeze-dried vaccine dissolves to give a clear or slightly opaque suspension. Withdraw the total amount of dissolved vaccine into the syringe and replace the long needle with the smaller needle for IM injection. The reconstituted vaccine should be used immediately.
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If either of these conditions exists, the vaccine should not be administered. A separate, sterile syringe and needle or a sterile disposable unit should be used for each patient to prevent transmission of hepatitis and other infectious agents from person to person. Needles should not be recapped and should be properly disposed of.
The lyophilization of the vaccine is performed under reduced pressure and the subsequent closure of the vials needs to be done under vacuum. Additionally, if there is no negative pressure in the vial, injection of Sterile Diluent for RabAvert would lead to an excess positive pressure in the vial. After reconstitution of the vaccine, it is recommended to unscrew the syringe from the needle to eliminate the negative pressure. After that, the vaccine can be easily withdrawn from the vial. It is not recommended to induce excess pressure, since over-pressurization will create the problems in withdrawing the proper amount of the vaccine.

HOW SUPPLIED

Package with:

1 vial of freeze-dried vaccine containing a single dose

1 vial of Sterile Diluent for RabAvert (1 mL)

1 disposable syringe

1 smaller needle for injection, 25 gauge × 1 "

1 longer needle for reconstitution, 21 gauge ×1.5 "

N.D.C.# 63851-501-01
CAUTION: Federal law prohibits dispensing without a prescription

Storage
RabAvert should be stored protected from light at 2°C to 8°C (36°F to 46°F). After reconstitution the vaccine is to be used immediately. The vaccine may not be used after the expiration date given on package and container.

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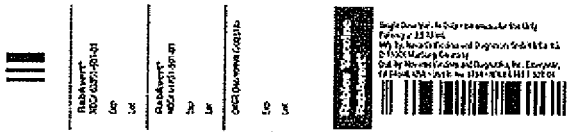

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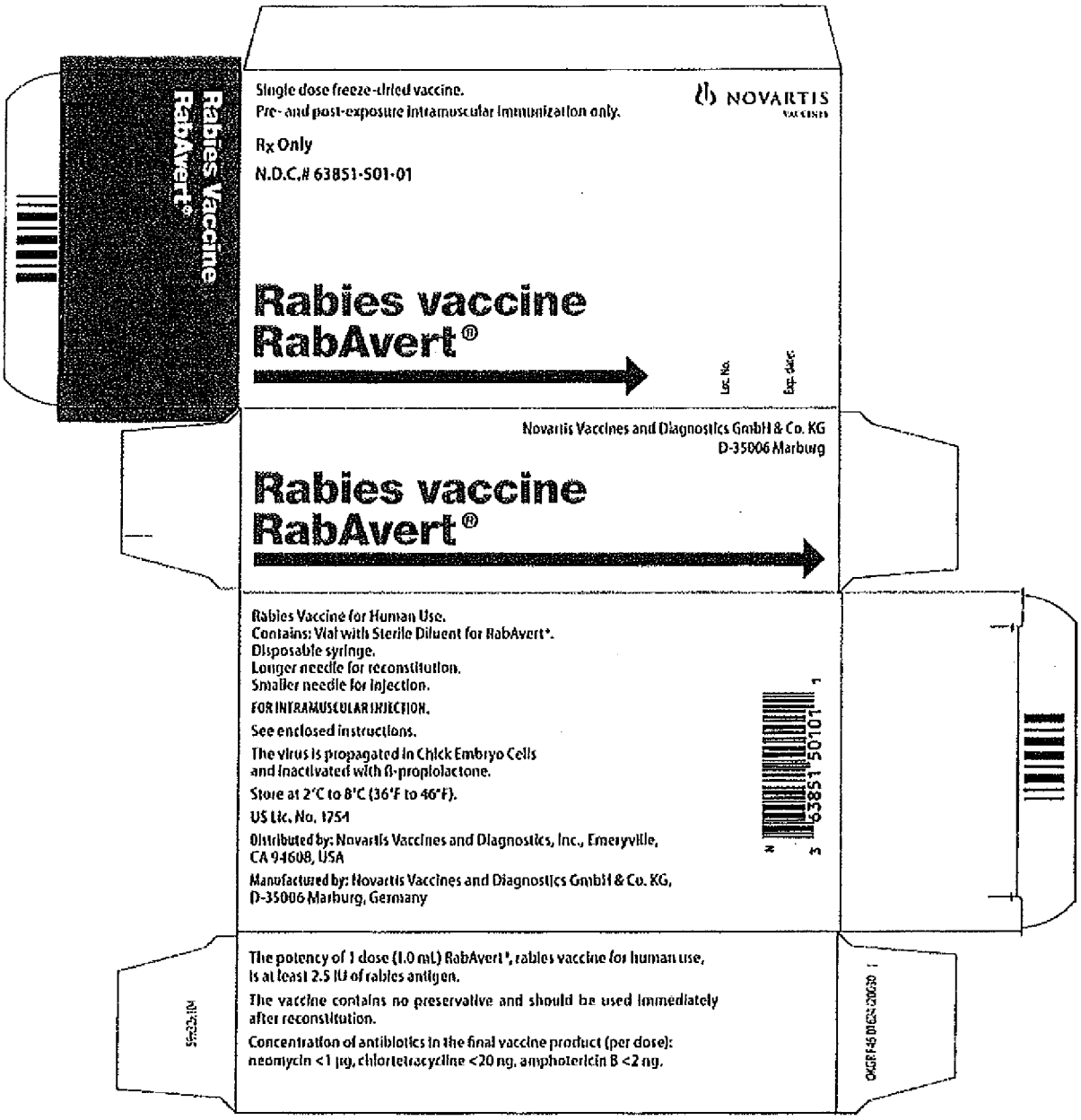
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FOR INTRAMUSCULAR INJECTION.
See enclosed instructions.
The virus is propagated in Chick Embryo Cells
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The potency of 1 dose (1.0 mL) RabAvert®, rabies vaccine for human use,
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The vaccine contains no preservative and should be used immediately
after reconstitution.
Concentration of antibiotics in the final vaccine product (per dose):
neomycin <1 µg, chlortetracycline <20 ng, amphotericin B <2 ng.